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Collis, J., Hill, M. R., Nicol, J. R., Paine, P. J., & Coulter, J. A. (2017). A hierarchical Bayesian approach to calibrating the linear-quadratic model from clonogenic survival assay data. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology*, 124(3), 541-546.
<https://doi.org/10.1016/j.radonc.2017.08.015>

Published in:

Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology

Document Version:

Peer reviewed version

Queen's University Belfast - Research Portal:

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A Hierarchical Bayesian Approach To Calibrating The Linear-Quadratic Model From Clonogenic Survival Assay Data.

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Word Count: 3210; Pages – 11; Figures – 5; Tables - 1

Key words: Bayesian hierarchical model, linear quadratic model, clonogenic assay, radiotherapy

Running title: Estimated LQ model parameters using a Bayesian hierarchical model.

Abstract

We propose a Bayesian hierarchical model applicable to the calibration of the linear-quadratic model of radiation dose-response. Experimental data used in model calibration were taken from a clonogenic survival assay conducted on human breast cancer cells (MDA-MB-231) across a range of radiation doses (0 Gy to 6 Gy). Employing Markov-chain Monte Carlo methods, we calibrated the proposed Bayesian hierarchical model, computed posterior distributions for the model parameters and survival fraction dose-response probability densities. Key contributions include the proposal of a model that incorporates multiple sources of inter- and intra-experiment variability commonly neglected in the standard frequentist approach and its subsequent application to *in vitro* experimental data.

1. Introduction

The clonogenic survival assay (first described in [1]) has become synonymous with determining cell survival response to ionising radiation. The assay measures the replicative differential between control and treated populations; based on the principle that a limited proportion of cells retain reproductive integrity and thus form viable colonies [2]. The ability of untreated cells to form colonies, referred to as the plating efficiency, is compared against treated groups in order to determine the surviving fraction (SF); where control cells are assigned an SF equal to one. Although these relatively simple *in vitro* assays lack the physiological complexity of *in vivo* tumours, their value in guiding clinical practise should not be underestimated. Variability in radiosensitivity is typically described by the shape of the radiation survival curve, allowing comparisons of intrinsic radiosensitivity, radiation quality, dose rate effects, fractionation protocols and the impact of environmental factors [3, 4, 5]. Importantly, while the predicted magnitude of these variables may be over- or underestimated using *in vitro* assays, important insights with translational relevance can be obtained.

For most clinical low linear energy transfer (LET) X-rays, radiation survival curves can be described in a qualitative manner as possessing two distinctive features, corresponding to lethal and sub-lethal lesions [6]. On a typical log-linear plot these components are evidenced by a linear reduction in survival as an exponential function of dose. As dose increases, though still within the range of clinically relevant fractions, the shape of the curve changes as the response to cell death resulting from the combined effect of the two components become significant (an effect proportional to dose squared) [6, 7]. In quantitative studies, this response is typically modelled using the linear-quadratic (LQ) model. The LQ model represents the most widely adopted model for predictions of radiation dose dependent effects. Applied to single fraction cell survival curves, the LQ model is given by

$$S(D; \alpha, \beta) = \exp(-(\alpha D + \beta D^2)), \quad (1)$$

where S denotes the fraction of cells surviving at dose D , α and β are model parameters [8]. Of clinical relevance is the dose where the contribution to cell death is equal between both linear and quadratic components, referred to as the α/β ratio [8, 9]. In the clinic, this value provides predictive capability for how tissue will respond to radiotherapy, helping to categorise both early and late responding tissue. In general, highly proliferative tumours have an α/β ratio greater than ten, leading to a linear reduction in cell survival at therapeutic doses; whereas a low α/β ratio (i.e. less than three) is characteristic of late responding tissue which requires higher dose fractions to achieve equivalent cell death [10, 11]. Determining the α/β ratio for each tumour type from simple *in vitro* clonogenic assays can, therefore, help identify optimal dose fractionation size in a tumour specific manner. However, despite widespread adoption of the LQ-formalism, concern exists relating to the accuracy of the model for high-dose hypofractionation protocols such those applied in stereotactic body radiotherapy (SBRT) [12, 13]. Pre-clinical data indicate that above a certain threshold dose, survival response becomes linear in contrast to the continual bending predicted by the LQ-model [14]. In practice the implication for LQ-based treatment plans is an underestimation of isoeffective doses for large radiation fractions. This underestimation in conjunction with imprecise assumptions of homogeneous radiobiology, variations in tumour density and the impact of the

microenvironment have necessitated the development of advanced predicative models based on LQ survival curves combined with Poisson based tumour control probability models, facilitated in part by functional imaging [15, 16].

We propose a Bayesian hierarchical regression model to estimate the parameters in the LQ model (1) for *in vitro* clonogenic data; a similar Bayesian approach was used for modelling *in vitro* dose-response data for a pharmaceutical agent [17]. It is beyond the scope of the current work to present a full treatment of Bayesian methods, and refer to recent tutorial articles [17, 18, 19]. Briefly, Bayesian calibration may be viewed as a means of updating subjective beliefs surrounding model parameters based on indirect experimental observations. The subjective nature of this approach naturally lends itself to assessing the probability of events in which the concept of repeated experiments under controlled conditions is flawed. For instance, assessing an individual patient's response to radiotherapy based on *in vitro* experiments of *ex vivo* tumour samples precludes truly identical repeated experiments as tumours exhibit significant heterogeneity. Moreover, inter-specimen variability in clonogenic assays introduces additional uncertainty in the utilization of these *in vitro* results to inform clinical decision-making. The hierarchical Bayesian approach adopted here may be used to model this variation and incorporate it into the predicted dose-response curves.

The focus of this work is to determine probability distributions for the model parameters employing a hierarchical Bayesian approach, with the goal of computing the probability density function (pdf) for the survival of cell populations under varying doses of irradiation, while comparing our Bayesian approach to the commonly used frequentist approach.

2. Materials and Methods

2.1 Experimental Methods

We obtained human breast cancer cells from the American Type Culture Collection (ATCC) and plated a total of 1.4×10^4 MDA-MB-231 cells in 35mm^2 dishes, supplemented with complete culture medium (DMEM+10% FBS) for 24 h. A schematic diagram showing the structure of the experiment is provided in Figure 1. The cells were irradiated at five dose levels, D_i , ($i = 1, \dots, 5$) from 0 Gy to 6 Gy (160 kVp X-rays), trypsinised, counted and re-plated at pre-determined low seeding densities. At each dose level, we repeated the assay twice, employing different numbers of seeded cells. Individual colonies then formed over 11 to 13 days in a humidified incubator at 37°C , in a mixture of 95% air and 5% CO_2 (total colonies formed represented by red and violet dots, Figure 1). Colonies were fixed and stained using 0.4% crystal violet, and counted three times ($n = 3$) to yield a mean colony count (counted colonies represented by violet dots, Figure 1). For the experiment analysed here, the assay was repeated three times ($m = 3$). Raw data (i.e. cell counts) obtained here may be found in [20].

2.2 Frequentist Analysis

We calculate plating efficiency, P , as the mean colony count divided by the number of seeded cells. We then average P obtained for each of the two repeat cell numbers to obtain the mean plating efficiency per experiment j . We compute the survival fraction, S_i^j , as the mean plating efficiency at a given non-zero dose D_i ($i \geq 1$) divided by mean plating efficiency at no dose (i.e. $D_1 = 0\text{Gy}$). The survival fraction per dose is then computed for each experiment. After completion of the above calculation, (1) was fit to the data employing the least squares method using the Python module *lmfit* [21]. In this approach, we consider two conceptual understandings of data collection and pooling between the experiments. In the first, we may view each experiment as independent, whereby we obtain distinct and independent parameter values α^j, β^j for each experiment j by analysing each experiment separately. In the second, we assume that the experiments are totally dependent, with a single set of parameter values α, β across all experiments obtained by analysing all data pooled together. We obtained a measure of goodness-of-fit, e.g., R^2 , and a confidence interval on the parameters α, β . These results are shown in Figure 2. Note that fitting the LQ model to the individual experiments (Fig. 2B), i.e., no pooling, gave spurious estimates for parameters α^j, β^j and confidence intervals (not shown), which is not very useful in predicting dose-response behavior.

Inevitably, there is some variation in our measured cell counts, though this is not explicitly propagated through to the survival fraction shown in Figure 2, as it is derived employing the mean values only. For example, in the first of the three experiments performed here, at 1.5 Gy, we obtain three counts (74, 74 and 89) for 200 initially seeded cells, and a further three (132, 110, and 116) for 400 initially seeded cells, yielding mean plating efficiencies of 0.395 and 0.298, respectively [20]. The average of these two values is then used to compute survival fraction for this experiment and dose level; and this technique is subsequently repeated for all other dose levels points and repeated experiments. Clearly, there is variation between the six colony counts at the two levels, though this variation is not explicitly propagated through to the survival fraction shown in Figure 2 as it is derived employing the mean values only. The Bayesian approach described below allows us to incorporate this uncertainty in our model predictions, and it allows for us to consider partial pooling, between the extreme cases described above.

2.3 Bayesian Hierarchical Model

We propose a Bayesian hierarchical model to assess the effect of dose on the proportion of cells surviving irradiation. In addition to inter-experiment variability, we consider measurement error between replicates and variability in cell survival and in colony formation. Thus, we may incorporate this intra-experiment variability into our model predictions. This is in contrast to the frequentist approach, in which this variability is lost when computing the plating efficiency using the mean colony count. Here, we do not compute P , but instead input directly all of the repeated colony counts into our model. The cell survival and colony formation is modelled by a Binomial distribution (i.e. each plated cell survives and forms a colony with a probability dependent on radiation dose and plating efficiency). The measurement of the colonies is modelled as a Poisson distribution (i.e. the number

of times a colony is counted is Poisson distribution with parameter λ). Finally, the weak inter-experiment dependence is modelled by considering the hyperparameters in the prior distributions for α^j and β^j as drawn from a common population distribution.

Given the LQ model (1) and the definition of plating efficiency P , we model the number of colonies formed C_i^j (violet and red dots in Figure 1) as binomially distributed, so that

$$C_i^j | \alpha^j, \beta^j, P^j \sim \text{Bin}(n_i^j, S(D_i; \alpha^j, \beta^j)P^j), \quad (2)$$

where $1 \leq i \leq l$ denotes the dose level, $1 \leq j \leq m$ denotes the experiment number and n_i^j is the (known) number of plated cells. During the measurement of the colonies the experimentalist may count each colony multiple times, or not at all. As such, we consider the total count of cells as the sum of C_i^j Poisson distributions with common parameter λ . Therefore, we model the number of colonies counted $N_{i,k}^j$ (violet dots in Figure 1) as Poisson distributed, so that

$$N_{i,k}^j | C_i^j, \lambda \sim \text{Po}(\lambda C_i^j), \quad (3)$$

where $1 \leq k \leq n$ denotes the replicant number.

We note that the same experimentalist conducted all counts in this study and, therefore, we naturally employ a common λ ($\lambda = 1$) throughout. However, if we considered data with multiple experimentalists conducting the count we may utilise multiple λ (potentially in a hierarchical manner) to assess the performance in the count.

The final stage in the construction of our model is to specify prior distributions on the model parameters α and β and the hyperparameters σ_α and σ_β , representing the respective variances of the model parameters. In contrast to the approach described in section 2.2, we incorporate partial pooling between experiments, allowing us to model the uncertainty in the regression coefficients of the different experiments, while informing these coefficients based on the hyperparameters describing their prior distributions as drawn from a common population distribution. When considering the plating efficiency, P , we discount interdependence between experiments and impose the prior distribution $\text{beta}(1.5, 2)$, which is informed from prior experience of conducting similar assays for this cell line. The biological intuition here is that it is natural to suppose that the posterior distributions for α and β should be identical for a given cell line and experimental setup. However, there are typically *a priori* unquantified variations that occur during the experiment. The population level hyperparameter distributions account for the level of similarity imposed between experiments (i.e. should we get a common α and β across all experiments; is there

complete independence between each experiment; or, do we expect that the values obtained in each experiment should be similar to some extent, though different in each experiment).

Given prior knowledge on the typical dose-response curves for this cell line [22], we chose weakly informative half-normal prior distributions on the parameters α^j and β^j , so that they are constrained to be positive and large values are unlikely:

$$\begin{aligned}\alpha^j &\sim HN(\sigma_\alpha^2) \\ \beta^j &\sim HN(\sigma_\beta^2).\end{aligned}$$

Due to our lack of *a priori* knowledge on the respective hyper-parameters, σ_α and σ_β , we imposed weakly informative half-Cauchy prior distributions in line with [23, 24]

$$\begin{aligned}\sigma_\alpha &\sim HC(\gamma) \\ \sigma_\beta &\sim HC(\gamma),\end{aligned}$$

where γ reflects the degree of pooling imposed on the values of α^j and β^j across the experiments, with $\gamma \rightarrow 0$ implying total pooling and $\gamma \rightarrow \infty$ implying no pooling. These priors were chosen to be weakly informative, but constrained so that $\alpha, \beta, \sigma_\alpha, \sigma_\beta \geq 0$. Further discussion regarding selection of priors for variance parameters in hierarchical models can be found in [24].

To perform the inference for the Bayesian hierarchical model we employ MCMC to generate posterior distributions of α and β in the LQ model, and their associated hyper-parameters (σ_α and σ_β). We refer to [25, 26, 27] and the references cited therein for a comprehensive discussion of Markov chains and MCMC. In order to assess whether the parameter space has been well-explored by the chain, and that it has converged to the equilibrium distribution we calculate the Gelman-Rubin scale reduction factor [28], where a value of less than 1.1 suggests that the chains have converged to a stationary distribution. The results of the convergence assessment are contained in Appendix A. The code used to run the MCMC scheme for the Bayesian hierarchical model described in this section is available in both R and Python, and can be found at [29]. In R we used the package rjags [30], which provides an interface to the JAGS MCMC library [31]. In Python, we used the statistical package pymc3 [32].

3. Results

Figure 2 shows the computed fit and 95% confidence intervals based on calibrating (1) adopting the frequentist approach described in Section 2.2. Figure 3 shows the posterior pdfs for the model parameters and associated hyper-parameters obtained from calibrating (1) using the hierarchical Bayesian approach set out in Section 2.3. Figure 4 shows the computed posterior median and 95 % credible region for dose-dependent survival. This plot was generated by drawing from the posterior distributions of α and β (i.e. the posterior distributions shown in Figure 3) computing survival fractions at a range of dose values based on (1). Figure 5 provides a comparison between frequentist and Bayesian approaches.

4. Discussion

We propose a Bayesian hierarchical model to estimate the radiation dose response of human breast cancer cells, employing experimental clonogenic survival data. The resulting model predictions account for the uncertainty in survival fraction for a given dose as a result of inter-experiment variation, common with the frequentist approach used in these assays. Additionally, the hierarchical Bayesian model we propose explicitly accounts for uncertainty in plating efficiency in an individual experiment and between replicates of cell count (intra-experiment variability). This hierarchical model allows us to quantify and utilise this variation in repeated measurements taken from both the control and the irradiated cell populations in our model prediction. Instead of confidence intervals, commonly reported with frequentist methods, we obtain posterior pdfs for the model parameters α^j and β^j for each experiment as shown in Figure 3. With the hierarchical Bayesian approach, we obtain a model that lies somewhere between the two frequentist approaches described in Section 2.2, resulting from the common population distributions employed in the selection of the prior distributions on the model parameters, noting we could easily alter the degree of pooling by selecting other values of γ .

Variation in dose response is reflected in the thickness of the shaded area in the plot of survival fraction against dose shown in Figure 4. Contrasting this against the results obtained from the frequentist approach shown Figure 2, we can remark that there is increased information regarding the uncertainty of the model predictions in our Bayesian model. In the frequentist approach, we have neglected multiple sources of uncertainty by averaging the repeated measurements; by using only the mean survival fraction per dose per experiment, the frequentist method only accounts for the inter-experiment variation, while the Bayesian approach accounts for uncertainty in cell counting and variations between replicates, in addition to the inter-experiment variation. The Bayesian approach offers a further advantage in that we can immediately calculate intervals that contain the parameters within a desired probability, instead of the less intuitive concept of confidence intervals typically employed with frequentist methods. In particular, it is straightforward to compute clinically relevant quantities of interest such as the probability that the survival fraction at a given dose is lower than a specified value (i.e. $p(S(D) < \text{Val})$).

The Bayesian approach used in this paper offers a large degree of flexibility, and although this requires the user to make a number of modelling decisions regarding priors and their hyper-parameters, there are well-established choices in the literature. An accessible tutorial for fitting Bayesian models, including a discussion of prior distributions, to a tumour growth model can be found in [15] and for an in-depth discussion of the choice of variance

parameters in prior distributions see [19]. The choice of priors and hyper-parameters should be informed as much as possible by expert knowledge and no one choice should be considered a panacea.

In Figure 2 and Figure 3 we obtain results consistent with parameter values reported in [22]. The posteriors for σ_α and σ_β shown in Figure 3 reflect the variation in the model parameters across the experiments. The posterior distributions for plating efficiency show more colonies formed in experiment 1 than in experiments 2 and 3, which is clearly shown by the positions of the pdfs in Figure 3. This inclusion of variations in plating efficiency in the computation of the survival fraction versus dose curve Figure 4 represents a key advantage of the Bayesian approach. The posterior distributions shown in Figure 3 are all unimodal, with mean values far from the imposed through the choice of prior; thus, vindicating our choice of prior.

To illustrate how results from the Bayesian approach may be used to inform clinical dosing levels, we computed the probability distribution of the α/β ratio utilizing random draws from the posterior distributions on α and β shown in Figure 5B. This distribution is well within the range of 0-3, indicating that a late response of these cells to radiation doses, suggesting that higher dose fractions should be used to treat this cell type. The width of this distribution, along with width of the survival fraction versus dose curve in Figure 4 could be indicative of the potential effectiveness of a treatment regime that was developed by utilizing the approach outlined in this article. Moreover, this approach may be applied to developing population-specific treatment strategies. The dose-response of biopsied cells taken from a patient population could be tested using the clonogenic survival assay. Our proposed hierarchical Bayesian model could then be applied to generate posterior distributions on α^j and β^j , this time per patient. From these, both a survival fraction versus dose curve and the distribution of α/β for the patient population could be generated. If point estimates are required to comply with current clinical practice, for instance in therapy planning, one could compute the median or mode for any of the posterior distributions presented above.

Acknowledgements

JC, JAC, and JRN acknowledge the support of EPSRC grant number EP/K039342/1. JC, MRH and PJP acknowledge informative conversations with Kirsty J. Bolton and John Pickton at the University of Nottingham.

Bibliography

- [1] T. Puck and P. Marcus, "Action of x-rays on mammalian cells," *J Exp Med*, vol. 103, no. 5, pp. 653-666, 1956.
- [2] N. Franken, R. HM, J. Stap, J. Haveman and C. van Bree, "Clonogenic assay of cells in vitro," *Nat Protoc*, vol. 1, no. 5, pp. 2315-2319, 2006.
- [3] E. Hall, Radiobiology for radiologist, Fourth Edition ed., Philadelphia: Lippincott Williams & Wilkins, 2000.
- [4] V. Prokic, P. Jacob and W. Heidenreich, "Possible implications of non-linear radiobiological effects for the estimation of radiation risk at low doses," *Radiat Prot Dosimetry*, vol. 99, no. 1-4, pp. 279-281, 2002.
- [5] M. Horsman and J. Overgaard, "The impact of hypoxia and its modification of the outcome of radiotherapy," *J Radiat Res*, vol. 1, pp. i90-i98, 2016.

- [6] G. Barendsen, "Mechanisms of cell reproductive death and shapes of radiation dose--survival curves of mammalian cells," *Int J Radiat Biol*, vol. 57, no. 4, pp. 885-896, 1990.
- [7] N. Franken, A. Oei, H. Kok, H. Rodermond, P. Sminia, J. Crezee, L. Stalpers and G. Barendsen, "Cell survival and radiosensitisation: modulation of the linear and quadratic parameters of the LQ model," *Int J Oncol*, vol. 42, no. 5, pp. 1501-1515, 2013.
- [8] R. Dale, "The application of the linear-quadratic dose-effect equation to fractionated and protracted radiotherapy," *Br J Radiol*, vol. 58, no. 690, pp. 515-528, 1985.
- [9] J. Fowler, "The linear-quadratic formula and progress in fractionated radiotherapy," *Br J Radiol*, vol. 62, no. 740, pp. 679-694, 1989.
- [10] D. Brenner, A. Martinez, G. Edmundson, C. Mitchell, H. Thames and E. Armour, "Direct evidence that prostate tumors show high sensitivity to fractionation (low alpha/beta ratio), similar to late-responding normal tissue," *Int J Radiat Oncol Biol Phys*, vol. 52, no. 1, pp. 6-13, 2002.
- [11] M. Wedenberg, B. Lind and B. Hårdemark, "A model for the relative biological effectiveness of protons: the tissue specific parameter α/β of photons is a predictor for the sensitivity to LET changes," *Acta Oncol*, vol. 52, no. 3, pp. 580-588, 2013.
- [12] J. P. Kirkpatrick, J. J. Meyer and L. B. Marks, "The Linear-Quadratic model is inappropriate to model high dose per fraction effects in radiosurgery," *Semin Radiat Oncol*, vol. 18, no. 4, pp. 240-243, 2008.
- [13] M. L. Guckenberger, R. J. Klement, M. Allgauer, S. Appold, K. Dieckmann, I. Ernst, U. Ganswindt, R. Holy, U. Nestle, M. Nevinny-Stickel, S. Semrau, F. Sterzing, A. Wittig, N. Andratchke and M. Flentje, "Applicability of the linear-quadratic formalism for modeling local tumor control probability in high dose per fraction stereotactic body radiotherapy for early stage non-small cell lung cancer," *Radiother Oncol.*, vol. 109, no. 1, pp. 13-20, 2013.
- [14] H. Iwata, Y. Shibamoto, R. Murata, N. Tomita, S. Ayakawa, H. Ogino and M. Ito, "Estimation of errors associated with use of linear-quadratic formalism for evaluation of biologic equivalence between single and hypofractionated radiation doses: an in vitro study," *Int J Radiat Oncol Biol Phys*, vol. 75, no. 2, pp. 482-488, 2009.
- [15] A. E. Nahum and J. Uzan, "(Radio)biological optimization of external-beam radiotherapy," *Comput math methods med*, vol. 2012, 2012.
- [16] O. Casares-Magaz, U. A. van der Heide, J. Rorvik, P. Steenbergen and L. P. Muren, "A tumour control probability model for radiotherapy of prostate cancer using magnetic resonance imaging-based apparent diffusion coefficient maps," *Radiother Oncol*, vol. 119, no. 1, pp. 111-116, 2016.
- [17] M. Allmaras, W. Bangerth, J. M. Linhart, J. Polanco, F. Wang, K. Wang, J. Webster and S. Zedler, "Estimating Parameters in Physical Models through Bayesian Inversion: A Complete Example," *SIAM Rev.*, vol. 55, no. 1, pp. 149-167, 2013.
- [18] O. Aguilar, M. Allmaras, W. Bangerth and L. Tenorio, "Statistics of Parameter Estimates: A Concrete Example," *SIAM Rev.*, vol. 57, no. 1, pp. 131-149, 2015.
- [19] J. Collis, A. Connor, M. Paczkowski, P. Kannan, J. Pitt-Francis, H. Byrne and M. Hubbard, "Bayesian calibration, validation and uncertainty quantification for predictive modelling of tumour growth: a tutorial," *Bull Math Biol*, 2017.
- [20] J. Collis, J. A. Coulter, M. R. Hill, J. R. Nicol and P. J. Paine, "Bayesian Calibration of LQ Model from Clonogenic Assay," 2017. [Online]. Available: https://figshare.com/articles/MDA-MB213_control/4635253. [Accessed 25 3 2017].
- [21] M. Newville, T. Stensitzki, D. Allen and A. Ingargiola, "LMFIT: Non- Linear Least-Square Minimization and Curve-Fitting for Python," 2014. [Online]. Available: <https://doi.org/10.5281/zenodo.11813>.
- [22] S. Jain, J. Coulter, A. Hounsell, K. Butterworth, S. McMahon, W. Hyland, M. Muir, G. Dickson, K. Prise, F. Currell, J. O'Sullivan and D. Hirst, "Cell- specific radiosensitization by gold nanoparticles at megavoltage radiation energies," *Int J Radiat Oncol Biol Phys*, vol. 79, no. 2, pp. 531-539, 2011.
- [23] V. Hennessey, G. Rosner, R. Bast and M.-Y. Chen, "A bayesian approach to doseresponse assessment and synergy and its application to in vitro doseresponse studies," *Biometrika*, vol. 66, no. 4, p. 1275-1283, 2010.
- [24] A. Gelman, "Prior distributions for variance parameters in hierarchical models," *Bayesian Analysis*, vol. 1, pp. 515-533, 2006.

- [25] W. Gilks, S. Richardson and D. Spiegelhalter, Eds., *Practical Markov Chain Monte Carlo*, London: Chapman and Hall, 1996.
- [26] S. Chib and E. Greenberg, "Understanding the Metropolis-Hastings algorithm," *The American Statistician*, vol. 49, no. 4, p. 327–335, 1995.
- [27] J. Norris, *Markov Chains*, Cambridge: Cambridge University Press, 1998.
- [28] J. Geweke, "Evaluating the accuracy of sampling-based approaches to calculating the posterior moments," in *Bayesian Statistics 4*, J. Bernardo, J. Berger, A. Dawid and A. Smith, Eds., 1992, p. 169–193.
- [29] J. Collis, M. R. Hill and P. J. Paine, "Bayesian Calibration of LQ Model from Clonogenic Assay," 2017. [Online]. Available: https://figshare.com/articles/Python_Script/4622389.
- [30] M. Plummer, A. Stukalov and M. Denwood, "rjags Package Documentation," 17 2 2017. [Online]. Available: <https://cran.r-project.org/web/packages/rjags/rjags.pdf>.
- [31] M. Plummer, "JAGS: Just Another Gibbs Sampler," [Online]. Available: <https://sourceforge.net/projects/mcmc-jags/files/?source=navbar>. [Accessed 17 2 2017].
- [32] J. Salvatier, T. Wiecki and C. Fonnesbeck, "Probabilistic programming in python using pymc3," *PeerJ Computer Science*, vol. 2, no. 55, 2016.
- [33] B. Marples, P. Lambin, K. Skov and M. Joiner, "Low dose hyper-radiosensitivity and increased radioresistance in mammalian cells," *Int J Radiat Biol*, vol. 71, no. 6, pp. 721-735, 1997.
- [34] A. Chalmers, P. Johnston, M. Woodcock, M. Joiner and B. Marples, "PARP-1, PARP-2, and the cellular response to low doses of ionizing radiation," *Int J Radiat Oncol Biol Phys*, vol. 58, no. 2, pp. 410-419, 2004.
- [35] F. McKenna and S. Ahmad, "Fitting techniques of cell survival curves in high-dose region for use in stereotactic body radiation therapy," *Phys Med Biol*, vol. 54, no. 6, pp. 1593-1608, 2009.
- [36] S. Unkel, C. Belka and K. Lauber, "On the analysis of clonogenic survival data: Statistical alternatives to the linear-quadratic model," *Radiat Oncol*, vol. 11, no. 11, 2016.
- [37] A. Gelman, J. Carlin, H. Stern, D. Dunson, A. Vehtari and D. Rubin, *Bayesian Data Analysis*, Third Edition ed., Boca Raton: CRC Press, 2014.
- [38] J. Kaipio and E. Somersalo, *Statistical and Computational Inverse Problems*, New York: Springer, 2006.
- [39] N. Metropolis, A. Rosenbluth, M. Rosenbluth, A. Teller and E. Teller, "Equation of state calculations by fast computing machines," *Journal of Chemical Physics*, vol. 21, pp. 1087-1092, 1953.
- [40] N. Metropolis and S. Ulam, "The Monte Carlo method," *J Amer Statist Assoc*, vol. 44, p. 335–341, 1949.

Figure 1.

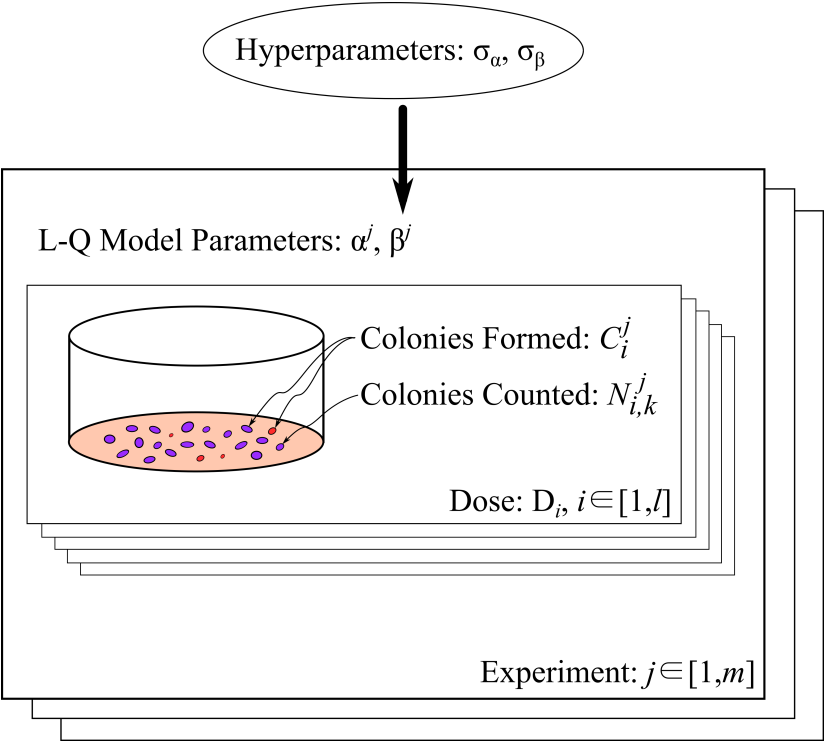


Figure 2.

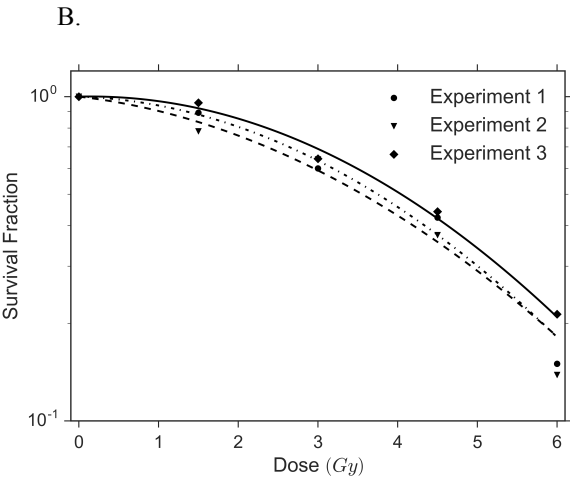
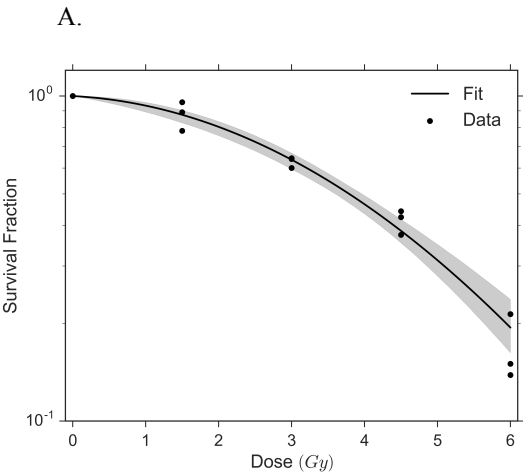


Figure 3.

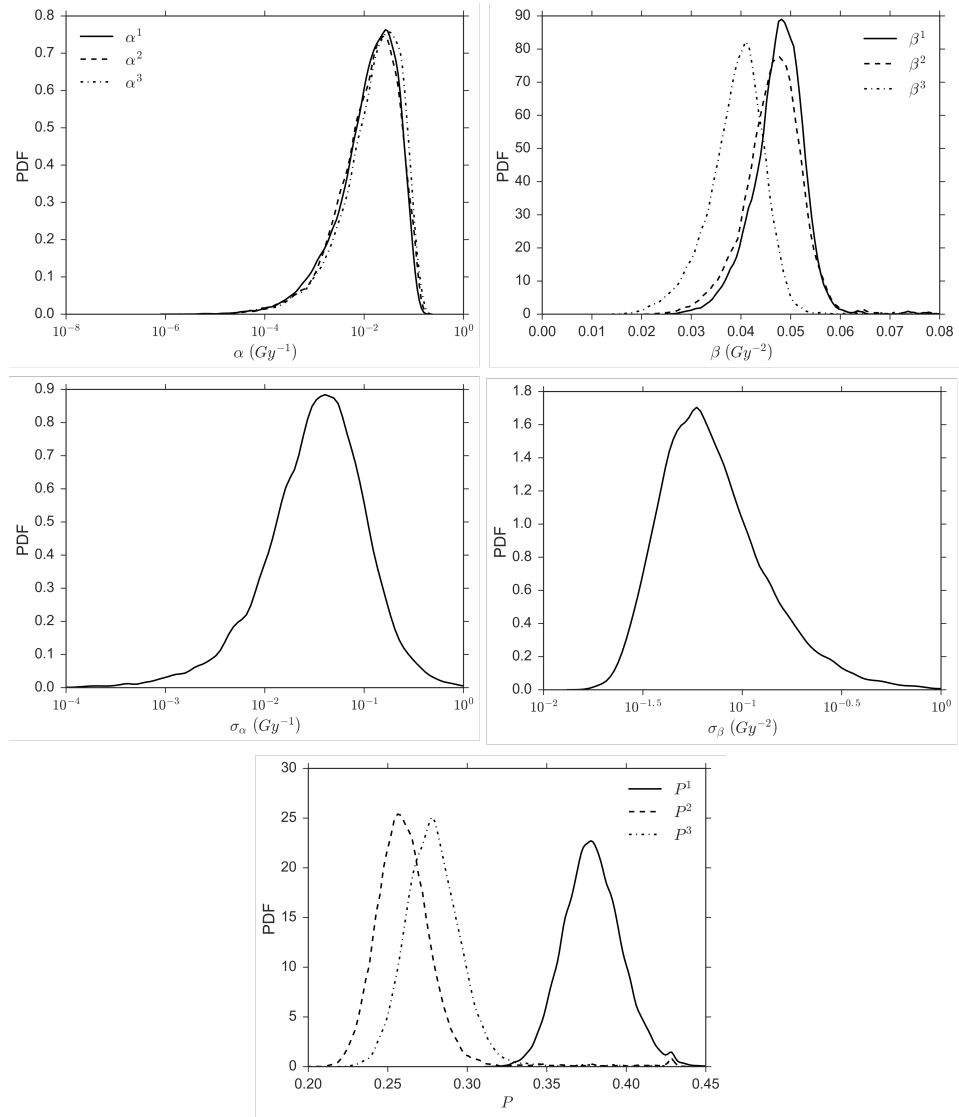


Figure 4.

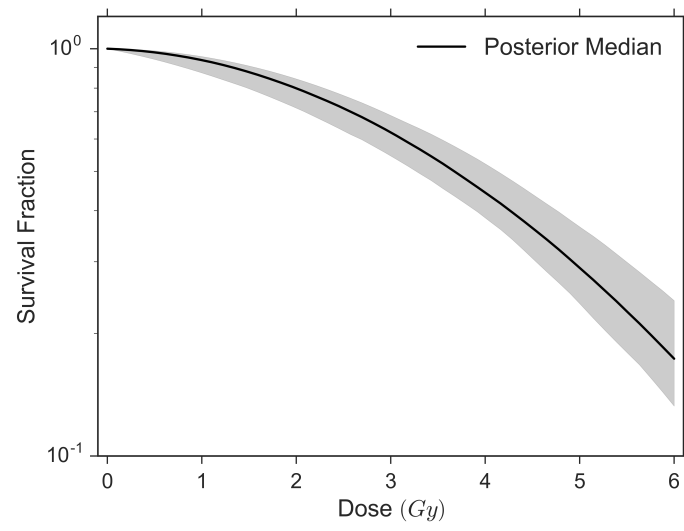


Figure 5.

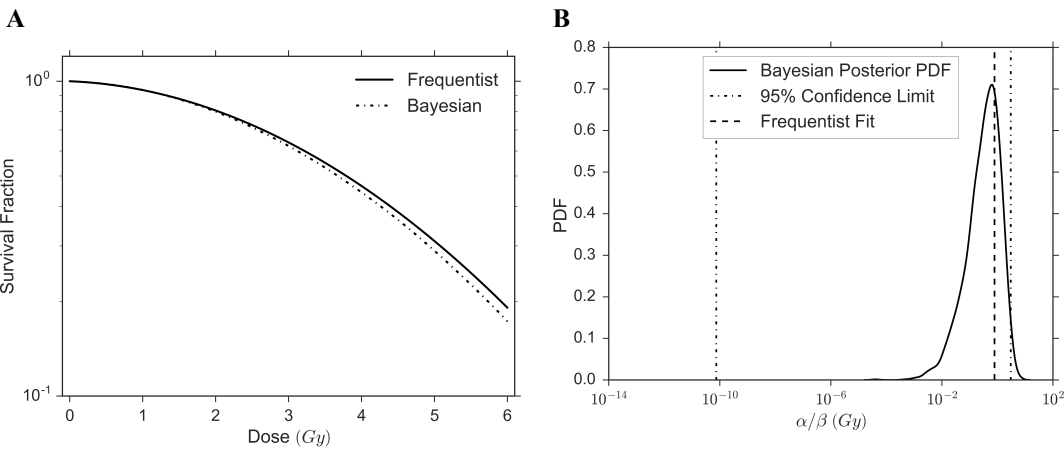


Figure 1. *Schematic diagram of the experimental structure and illustration of the Bayesian hierarchical approach.* Linear-quadratic (LQ) model (1) parameters α^j and β^j are given per experiment j . Total colonies formed, C_i^j , modelled by a binomial distribution (2), are depicted as red and violet dots, with the latter representing colonies counted by the experimenter, $N_{i,k}^j$, represented by a Poisson distribution (3), noting some colonies may not be counted and some may be counted more than once. Repeated counts are made by the experimenter, where $k \in [1, n]$ denotes replicant number. Hyperparameters σ_α and σ_β represent variances in the model parameters. For the given data, $l = 5, m = 3, n = 3$.

Figure 2: A) Survival fraction versus radiation dose, showing raw data and fit of (1) to the pooled data, using least-squares regression, with shaded area depicting the 95 % confidence interval. $R^2 = 0.981$, and best-fit parameter values with 95 % CI are: $\alpha = 0.0223 \pm 0.0458$ and $\beta = 0.0422 \pm 0.0118$. B) Survival fraction versus radiation dose obtained by fitting the data obtained in each experiment separately, i.e. employing un-pooled data.

Figure 3: Posterior distributions of the parameters and hyperparameters; α^j , β^j , σ_α , σ_β and P^j .

Figure 4: Posterior median of the survival curve obtained employing the Bayesian approach with shaded area depicting a 95 % credible region.

Figure 5: A) Comparison of survival curves obtained using the frequentist and Bayesian approach. **B)** Posterior distribution of the α/β ratio pooled across the three experiments. This plot shows the Bayesian marginal pointwise posterior median survival fraction and its corresponding frequentist MLE together with 95% confidence interval.